REMARKS

Claims 1-35 are pending in the present application. After restriction, claims 3, 5-7, 9-10, 12-15, and 18-35 are withdrawn. Claims 1, 2, 4, 8, 11, 16 and 17 (Group I) are the subject of the present Office Action. The Examiner has acknowledged that claim 1 was inadvertently not included in Group I in the restriction requirement of July 23, 2002.

Claims 1-3 and 24 have been amended. The specification has been amended to correct typographical errors and to insert sequence identification numbers. Support for amended claims 1-3 and 24 is found in the specification, e.g., at ¶ 61, final sentence; ¶ 114, second sentence; and ¶ 115, third sentence. No new matter has been introduced. Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Applicants acknowledge the Examiner's statement that Groups VIII (claims 26-28) and IX (claim 29-35) are method claims that incorporate all of the limitations of composition claims contained in Group I, and that should the Examiner find any of these claims allowable, the Examiner will rejoin the methods claims of Groups VIII and IX to the extent they incorporate all the limitations of allowed composition claims of Group I. Applicants respectfully reiterate the arguments presented in the response mailed September 20, 2002 (paper 11) with respect to rejoinder of Groups II, III, and IV.

Applicants expressly reserve their right under 35 U.S.C. § 121 to file a divisional application directed to the nonelected subject matter during the pendency of this application, or an application claiming priority from this application.

Claim 8 is objected to under 37 CFR 1.75 (c).

Applicants respectfully traverse the objection. Claim 8 is in proper dependent form, because it is in the alternative form, and because it does not depend from a multiple dependent claim. See MPEP § 608.01(n). Multiple dependent claims are clearly permissible. Thus, Applicants request that the objection be withdrawn.

Claims 4, 16 and 17 stand rejected under 35 U.S.C. 112, second paragraph.

Claims 4, 16, and 17 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter which Applicants regard as the invention.

Applicants respectfully traverse the rejection. Claims 4, 16 and 17 are definite under the test for definiteness under 35 U.S.C. § 112, second paragraph, which is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576 (Fed. Cir. 1986)

Claim 4 is directed to fusion proteins of alpha 1-antitrypsin (AAT) or a functionally active portion thereof and secretory leukocyte inhibitor (SLPI) or a functionally active portion thereof, where the fusion protein contains amino acids from about 1 to about 394 of AAT and amino acids from about 1 to about 107 of SLPI. Claims 16 and 17 recite that the carboxy terminus of AAT is linked to the amino terminus of SLPI, or that the carboxy terminus of SLPI is linked to the amino terminus of AAT.

The Office has stated that one of skill in the art would not know from which sequences to select amino acids 1-394 or 1-107 without a sequence identification number. It is not necessary to restrict the claim to a single sequence in order for a person of ordinary skill to interpret the metes and bounds of the claim. One of skill in the art, reading the claim in view of the specification, would understand "AAT" or "SLPI" to mean human as well as other AAT and SLPI, as well as any variants of these AAT's and SLPI's. See, e.g., specification paragraphs 59, 67, and 68. The sequences of recombinant and/or natural variants within or between species are

known. For AAT variants, see, e.g., Patterson, S.D., Mammalian alpha 1-antitrypsins: comparative biochemistry and genetics of the major plasma serpin, *Comp. Biochem. Physiol. B* 100:439-454 (1999); Jeppsson and Laurell, The amino acid substitutions of human alpha 1-antitrypsin M3, X and Z, *FEBS Lett.*231:327-330 (1988); Crystal, *et al.*, The alpha 1-antitrypsin gene and its mutations. Clinical consequences and strategies for therapy. *Chest 95: 196-208*, Luisetti, M., Alpha 1-antitrypsin variants produced by recombinant DNA: differences in elastase inhibitory activity and resistance to oxidant agents. *Int. J. Tissue React.* 12:363-368, (1990); Kwon, *et al.*, Single amino acid substitutions of alpha 1-antitrypsin that confer enhancement in thermal stability. *J. Biol. Chem.* 269:9627-9631 (1994). For SLPI variants see, e.g., U.S. Patent No. 5,871,956, col. 12, lines 6-33; U.S. Patent No. 4,760,130, claims 1-9. One of skill in the art may use a database of protein sequences and/or a database of the scientific literature to determine what sequences are covered by the claims.

it is not required

In addition, claims 4, 16 and 17 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for reciting the term "about." Specifically, the Office asserts that "about" is a relative term which renders the claim indefinite. Applicants respectfully traverse the rejection.

Applicants respectfully submit that the use of the term "about" does not render claims using this term indefinite. It is well established that the use of a relative term does not render a claim indefinite under 35 USC § 112, second paragraph. *See Seattle Box Co. v. Industrial Crating & Packaging, Inc.*, 731 F.2d 818, 221 USPQ 568 (Fed. Cir. 1984) (stating that the fact that the claim language, including terms of degree, may not be precise, does not automatically render the claim indefinite); *see also* U.S. Patent & Trademark Office, *Manual of Patent Examining Procedure* § 2173.05(b). Claims are definite where "the claims, read in light of the specification, reasonably apprise those skilled in the art and are as precise as the subject matter permits. As a matter of law, no court can demand more." *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 USPQ 81, 94, 95 (Fed. Cir. 1986).

decide

Moreover, the term "about" is accepted and widely used in patent practice and is clearly acceptable under the law. The word "about" does not have a universal meaning in patent claims; rather, its meaning depends on the technological facts of the particular case. *Pall Corp. v. Micron Separations, Inc.*, 66 F.3d 1211, 1217-18 (Fed. Cir. 1995); *see also* U.S. Patent & Trademark Office, *Manual of Patent Examining Procedure* § 2173.05(b). "About" is neither broad nor arbitrary, but rather serves as a flexible term with a meaning similar to "approximately." *Conopco, Inc. v. May Dep't Stores Co.*, 46 F.3d 1556, 1561 (Fed. Cir. 1994); *see also Ex parte Eastwood*, 163 USPQ 316 (Brd. App. 1968). In *Hybritech, supra,* the limitation "at least about 10^8 liters/mole" was found to be definite in view of the specification and the inexact nature of the subject matter. *Id.* Similarly, in *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557 (Fed. Cir. 1983), the phrase "exceeding about" was found to be definite, and in *Modine Mfg. Co. v. Int'l Trade Comm'n*, 75 F.3d 1545, 37 USPQ2d 1609 (Fed. Cir. 1996), the phrase "about 0.015-0.040" was found to be definite. *See Modine Mfg.*, 721 F.2d at 1545 (stating that "mathematical precision should not be imposed for its own sake; the patentee has the right to claim the invention in terms that would be understood by persons of skill in the art").

As a further example, Applicants direct the Office's attention to issued U.S. Patent Nos. 6,242,570; 6,242,214; 6, 231,864; 6,235,716; 6,235,714; 6,228,825; 6,228,825; 6, 228, 983; 6,225,442; 6,217,864; and 6,214,539, in which the word "about" is used in the claims to describe a length of a claimed polypeptide or a range of amino acids within a peptide. In addition, a search of issued U.S. patents (searching for the term "amino acid" with 5 words of the term "about") revealed 175 issued U.S. patents since October 1, 2000 in which the term "about" is used in the claims to describe either a range of amino acids, or the length of a claimed polypeptide.

Applicants submit that the use of the word "about" in the present application is acceptable under the law, and, in view of the disclosed subject matter, the specification, and the cited caselaw, it is entirely appropriate to describe the length of the constituent peptides of the

claimed fusion proteins of AAT and SLPI with the word "about." Applicants respectfully request a withdrawal of this ground of rejection of claims 4, 16, and 17.

Claims 1, 2, and 11 stand rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking written description.

The Office has rejected claims 1, 2, and 11 as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Examiner acknowledges that Applicants teach several representatives of the genus wherein the species have the function of protease inhibitors and structure described by amino acid sequences of SEQ ID NOS: 8, 16, 10, 18, 14, 20, and 22, but contends that this is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. The Examiner also states that the specification fails to teach a structure function relationship for the claimed fusion proteins.

Applicants respectfully traverse this rejection.

Applicants respectfully submit that the specification provides ample description for claims 1, 2, and 11. In *The Regents of the University of California v. Eli Lilly*, 119 F.3d 1559 (Fed. Cir. 1997), the court affirmed that "every species in a genus need not be described in order that a genus meet the written description requirement." *Id.* at 1568. The court stated that description of a genus of polynucleotides "may be achieved by means of a recitation of a representative number of [polynucleotides], defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." *Id.* at 1569.

Applicants respectfully submit that the present claims are supported by both a representative number of polypeptide sequences falling within the scope of the genus and a

recitation of structural features common to the members of the genus. As acknowledged by the Examiner, Applicants teach several representatives of the genus wherein the species have the function of protease inhibitors and structure described by amino acid sequences of SEQ ID NOS: 8, 16, 10, 18, 14, 20, and 22. This selection by the Examiner encompasses fusion proteins, but one of skill in the art is apprised of Applicants' possession of the invention as claimed by the numerous sequences of protease inhibitors, and functionally active portions of protease inhibitors, that may serve as components of the fusion proteins of the invention. In addition to an amino acid sequence of human AAT (SEQ ID NO: 2), the specification provides the amino acid sequences of SLPI (SEQ ID NO: 4), the N-terminal portion of tissue inhibitor of metalloproteases, with and without an initial methionine (N-TIMP, SEQ ID NOS: 22 and 24, respectively), and pepstatin, which is an aspartyl protease (SEQ ID NO: 12). A large number of other protease inhibitors of the various classes are also described in the specification by reference to their published sequences, including general references to reviews of protease inhibitors (see paragraph 60); descriptions of the serine protease inhibitor superfamily, including the conserved domain of 370-390 residues (paragraph 66); AAT (paragraph 67); SLPI, including several patents that contain sequences of functionally active portions of SLPI (paragraph 68); tissue inhibitors of matrix metalloprotease inhibitors (TIMP's), including references to the amino acid sequences of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (paragraph 70) and functionally active portions of TIMP's (paragraph 71); cysteine proteases, including the conserved β-hairpin loop of cystatins and stefins (paragraph 73); and aspartyl protease inhibitors, including not only pepstatin (see SEQ ID NO: 12, above) but statin-like aspartyl protease inhibitors and other aspartyl protease inhibitors (paragraph 76). Hence every class of protease inhibitors is described, either by amino acid sequence in the specification, or by, generally, several sequences provided by reference. The structure of the linkage between the peptides of the fusion protein is also described (paragraph 69).

The specification also provides a description of structural features common to members of the genuses of claims 1, 2, and 11. All of the claims recite that the fusion protein contains

AAT or a functionally active portion thereof. AAT is amply described in the specification as detailed above. In addition, common structural features of the various classes of protease inhibitors included as the second protease inhibitor in the fusion proteins encompassed by the invention are also described, as detailed above.

From these structural descriptions one of skill in the art would reasonably conclude that Applicants had possession of the claimed genus, whether the genus is that of claim 1, 2, or 11.

The claims as amended recite that the claimed fusion proteins have protease inhibitor activity, and thus the genuses of claims 1, 2, and 11, as amended, do not encompass fusion proteins that do not have the desired characteristics, i.e., they are not protease inhibitors (see Office Action, pp. 6 and 7). In addition, numerous functionally active portions of protease inhibitors are described in the specification and are well-known in the art. The specification describes the N-terminal portion of tissue inhibitor of metalloproteases, with and without an initial methionine (N-TIMP, SEQ ID NOS: 22 and 24, respectively) and their use to construct fusion proteins with protease inhibitor activity (N-TAPI and rN-TAPI, Examples 2 and 3); numerous functionally active portions of SLPI, i.e., U.S. Patent Nos. 4,760,130; 5,464,822; 4,845,076; 5,633,227; 5,851,983; 5,871, 956; 5,900,400; 6,017,880; and 6,291,662, cited in paragraph 68; and numerous functionally active portions of TIMP's, i.e., Willenbrock *et al.*, *Biochemistry* 32: 4330-4337, 1993, Murphy, et al. *Biochemistry* 30: 8097-8102, 1991, Woessner, J. *FASEB J* 5: 2145-2154, 1991, and EPA publication # 0648838 A1, cited in paragraph 71. In addition, other functionally active portions of protease inhibitors, such as of AAT, are well-known and amply described in the literature; see, e.g., Niemann, M.A. *Biochim Biophys Acta* 1340:123-130 (1997). Thus, the structure function of a large number of active fragments of protease inhibitors useful in the invention are taught; indeed, the construction functionally active fusion proteins containing active fragments of tissue inhibitor of metalloproteases (N-TAPI and rN-TAPI) is taught, contrary to the Examiner's assertion that active fragments are not taught (Office Action pp. 6, 7).

Applicants also respectfully submit that when, as in the instant specification, there is ample and specific description of the structure of a number of species within a genus, there is no requirement for the specification to teach a "structure-function relationship" for the claimed components of structures of the genus, in this case, fusion proteins of protease inhibitors. The components of the fusion proteins encompassed by the present invention are known and are adequately, indeed, amply described by the amino acid sequences and structures taught in the instant specification. No more is required by statute or by the courts.

In view of the above, Applicants respectfully request withdrawal of this rejection.

Claims 1, 2, and 11 stand rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking enablement.

The Office contends that the specification does not reasonably provide enablement for fusion proteins encompassing proteins that include AAT and a second protease inhibitor, where the second protease inhibitor is any protease inhibitor (claim 1), any SLPI (claim 2), or any serine protease inhibitor (claim 11). Applicants respectfully submit that a *prima facie* case of enablement has not been made.

For a *prima facie* case on non-enablement, the burden is on the Office to demonstrate that there is a reasonable basis to question the presumptively sufficient disclosure made by applicant. See, e.g., *In re Wright*, 27 USPQ2d 1510 (Fed. Cir. 1993); MPEP § 2164.04. Absent evidence to the contrary, the specification must be assumed to be enabling. Applicants respectfully submit that a *prima facie* case of enablement has not been made.

The Examiner has noted that the specification is enabling for the fusion proteins of SEQ ID NOS: 8, 16, 10, 8, 14, 20, and 22. Applicants respectfully point out that it is a well-established principle of patent law that "patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991). In *In re Angstadt*, the Court of Customs and Patent Appeals considered the issue of whether section 112 requires disclosure of a test with every species covered by a claim and concluded that requirement of such a complete disclosure would necessitate a patent

application with thousands of examples and "would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments." *In re Angstadt*, 537 F.2d 498, 502 (CCPA 1976). The court concluded that such a requirement would be against public policy because it would have the effect of "depriving inventors of claims which adequately protect them and [would limit] them to claims which practically invite appropriation of the invention while avoiding infringement[, which would] inevitably [have] the effect of suppressing disclosure." *Id.* at 504. Based on the foregoing, Applicant is not required to disclose a working example of every fusion protein covered by the claim or even any additional working examples.

The Office states that undue experimentation would be required to make the claimed invention, citing the factors summarized in *In re Wands* (858 F.2d 731, Fed. Cir. 1988), namely, (a) the nature of the invention; (b), the breadth of the claim; (c) the state of the prior art; (d) the relative skill of those in the art; (e) the predictability of the art; (f) the presence or absence of working example; (g) the amount of direction or guidance presented; and (h) the quantity of experimentation necessary. The factors must all be considered in an enablement analysis; no one factor is dispositive. See, e.g., *Wands*, 740 ("Considering *all of the factors*, we conclude that it would not require undue experimentation . . . to practice the claimed invention." (Emphasis added.). See also MPEP § 2164.01(a), discussed more fully below.

In the Office Action, the Examiner repeatedly refers to quantity of experimentation (factor (h)), but does not analyze the enablement of Applicants' invention in terms of the other factors,[1] as required by the court's decision in *In re Wands* and the MPEP. A rejection based on one or two of these factors alone, while ignoring the other factors, is improper. When each of the factors is analyzed, the present claims are clearly enabled.

The Office has conceded that "[T]he knowledge of production of fusion protein is well developed [factors (a), (c) and (e)] and skills of artisans high [factor (d)]." Office Action, page 9.

The Office ignores the fact that the specification contains ample working examples (factor (f)) of fusion proteins of the invention; see Examples 1 (SLAPI and TAPI, which are fusion proteins of AAT and, respectively, SLPI and TIMP-1), Example 2 (N-TAPI, which is a fusion protein of AAT and a portion of TIMP-1, demonstrating the principle of using

functionally active portions of protease inhibitors), and Example 3 (r-NTAPI, r-TAPI, and r-SLAPI, which are fusion proteins in the reverse orientation, demonstrating the principle of using different termini of the component peptides for fusion).

The Office also ignores factor (g), the amount of direction or guidance presented. The present specification presents detailed and specific guidance on how to make the fusion proteins of the invention and how to test them for the requisite activity. This guidance is found in the body of the specification, which discusses at length and with specificity the various protease inhibitors that may be used in the invention (pp. 23-28 and 30-42), methods of producing the polynucleotides that code for the proteins (pp. 42-48), determining whether a given portion of a protease inhibitor is a "functionally active portion," including reference to generally-accepted algorithms of the art (pp. 28-30), construction of vectors (pp. 48-50), suitable host cells (p. 51), transfection of host cells (p. 52), and assays for activity of the various classes of protease (pp. 52-54). As noted, the Examples further provided step-by-step guidance in the construction and testing of ample specific examples of such fusion proteins.

The Office focuses only the two remaining Wands factors, the breadth of the claims (b) and the quantity of experimentation required (h). In terms of the breadth of the claims, the Office states that "The scope is so broad that it includes fusion proteins that have no desired functionality of being a protease inhibitor." Office Action, page 9. Applicants note that the claims have been amended to clarify that the claimed fusion proteins have protease inhibitor activity. Thus the only remaining Wands factor that the Office has analyzed in support of the present rejection is that of quantity of experimentation required. This factor will be addressed more fully below, but Applicants respectfully point out that, even if the Office were correct in it analysis of this factor, MPEP § 2164.01(a) states that "[i]t is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others." Hence, the present rejection based on alleged necessity for undue experimentation is improper under the current U.S.P.T.O. guidelines.

However, Applicants contend that even the quantity of experimentation necessary is not excessive, given the state of the art. The Office has asserted that "to make the invention one skilled in the art has to construct an enormous number of DNA molecules encoding fusion

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[1] The Office refers to breadth of the claims (Office Action, page 9), but the claims have

proteins generally described in claims 1, 2, and 11, express them, examine the inhibitory action of the fusion proteins obtained and select the ones that have the desired function. Thus, the experimentation necessary to make the invention is out of the realm of routine experimentation." Office Action, page 10. Applicants note that the specification has provided precise and detailed guidelines for the construction of fusion proteins of AAT and SLPI (claim 2), AAT and another serine protease inhibitor (claim 11), and AAT and another protease inhibitor (claim 1). The Examples contain step-by-step guidance for the construction and testing of SLAPI (a fusion of AAT and SLPI, both of which are serine protease inhibitors) and TAPI (a fusion of AAT and TIMP-1, a metalloprotease inhibitor). Thus much experimentation has already been done by the Applicants and disclosed to the public in the specification.

Furthermore, the steps that the Office has listed are routinely carried out by graduate students and, indeed, by undergraduate students, all over the world. While there may be a large number of species that could be constructed and tested, the requirements of doing so are well within the realm of "routine experimentation." The possibility that there may be a large quantity of experimentation required does not mean that the experimentation is undue. For example, in *Wands*, the court stated that even if only 2.8% of the constructs described by the specification were active, this did not necessarily mean that the claim for the constructs was not enabled. See *Wands*, 740, FN 29. See also, e.g., *Ex parte Jackson*, 217 U.S.P.Q. 804 (Bd. Pat. App. 1982) ("The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed.").

Taken together, the Wands factors indicate that the present invention is enabled. As the Office has conceded, the knowledge of production of fusion protein is well developed [factors (a), (c) and (e)] and skills of artisans high [factor (d)]. The specification contains ample working examples of fusion proteins of the invention (f), and presents detailed and specific guidance on how to make the fusion proteins of the invention and how to test them for the requisite activity (g). The claims as amended are directed to those fusion polypeptides having protease inhibitor activity (e). Finally, the quantity of experimentation is not excessive given the state of the art (h).

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been amended to reduce the breadth, as discussed more fully herein.

The Applicants respectfully submit that the Office has not produced any evidence to establish that a person skilled in the art could not make and use a fusion protein of AAT with another protease inhibitor, a serine protease inhibitor, or SLPI, as claimed without undue experimentation, given the teachings in the specification and the knowledge of those of ordinary skill in the art. Thus, the Office has failed to establish a *prima facie* case of non-enablement. Applicant's specification provides a presumptively sufficient disclosure providing ample teachings to allow a person skilled in the art to make and/or use the invention without undue experimentation. Applicant respectfully submits that the presently claimed invention is in compliance with enablement requirements.

In view of the above, Applicant respectfully requests withdrawal of this rejection.

CONCLUSION

Applicants have made a sincere effort to overcome the rejections and address all issues that were raised in the outstanding Office Action. Accordingly, reconsideration and allowance of the pending claims are respectfully requested. If it is determined that a telephone conversation would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 36829-2000200.

Respectfully submitted,

Dated: March 3, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph 71, bridging pages 38-39, has been amended as follows:

(Amended)

The complete structure of a TIMP is not necessarily a requirement for metalloprotease inhibition. For example, functionally active portions of TIMP-2 (often referred to as "TIMP-2 analogs") have been prepared that retain their inhibitory activity toward metalloproteases (see Willenbrock *et al.*, Biochemistry 32: 4330-4337, 1993). A partial sequence of TIMP-1 that contains only the first three loops of the molecule is capable of inhibiting matrix metalloproteases. (Note: the words "metalloprotease" and "metalloproteinase" are synonymous and both are used when referring to these enzymes in the literature; for consistency we will use only "metalloprotease" herein). The N-terminus of the TIMP molecule is where the inhibitory activity is found, and the inhibitory mechanisms appear to involve several specific amino acid sequences. (see, for example, Murphy, G. Houbrechts, A., Cockett, M.I., Williamson, R.A., O'Shea, M., and Docherty, AJP, The N-terminal domain of TIMP retains metalloprotease activity. *Biochemistry* 30: 8097-8102, 1991; Woessner, J., Matrix metalloproteases and their inhibitors in connective tissue remodeling. *FASEB J* 5: 2145-2154, 1991, and EPA publication # 0648838 A1, Tissue inhibitor of metalloprotease type three (TIMP-3), Silbiger and Koski). One preferred N-terminal fragment of TIMP-1 for construction of some embodiments of the present invention is the first 126 N-terminal amino acids of the native form. In making constructs this fragment is often used with an initial methionine, and thus contains 127 amino acids (the initial methionine plus the N-terminal 1-126 amino acids of TIMP-1); this fragment is referred to as N-TIMP 1-127 (see SEQ ID NO: 22 and Table ~~30~~ 28). Another preferred N-terminal fragment of TIMP-1 for construction of other embodiments of the present invention is the first 127 N-terminal amino acids of the native form. Amino acid 127 of this fragment is a free cysteine, and is thus available to participate in disulfide bond formation, which is one manner of constructing

the fusion proteins of the invention. In making constructs this fragment is often used with an initial methionine, and thus contains 128 amino acids (the initial methionine plus the N-terminal 1-127 amino acids of TIMP-1); this fragments is referred to as N-TIMP 1-128 (see SEQ ID NO: 24 and Table ~~32~~ 30).

Paragraph 174, bridging pages 108-109, has been amended as follows:

(Amended)

Example 8: Assay for AAT Activity Using Human Neutrophil Elastase.

Materials and Equipment:

1. *Human Neutrophil Elastase* (HNE), Lot # EH2000-2a from Athens Research & Technology: provided as a salt-free lyophilized powder containing 100μg protein; reconstituted with 200μL 50mM Na Acetate, pH 5.5, with 150 mM NaCl; divided into 20μL aliquots (16.9μM) and stored in -20°C

2. *Suc-Ala-Ala-Pro-Val-pNA* (SEQ ID NO: 33) (a chromogenic substrate for HNE), from Bachem: provided as a lyophilized powder containing 50mg peptide; reconstituted with 10mL DMSO (8.67mM); Divided into 500 μL aliquots and stored in –20°C.

3. *Activity assay buffer*: 0.1M Tris-HCl, 0.5M NaCl, pH 7.5, stored at room temperature

4. *96 wells Microtiter plates*, Catalog No.3474 from Costar—Ultra Low Cluster

5. *Multi-channel pipettor* from VWRbrand, 5-50μL or equivalent

6. *Multi-channel pipettor* from VWRbrand, 50-300μL or equivalent

7. *Microtiter Plate Reader Versa$_{max}$* from Molecular Devices

Methods:

1. The internal temperature of the Microtiter Plate Reader was set to 30°C and allowed to equilibrate at this temperature. Wavelength of the Reader was set at 405nm.

2. All testing was performed in triplicate. The three results were averaged.

3. The HNE Standard Curve was constructed in the range of 2.5nM to 20nM reaction concentration.

4. The HNE sample was diluted in activity assay buffer to 80nM, 40nM, 20nM, 10nM (HNE Standards).

5. Substrate Solution was prepared by diluting 8.67mM original stock to 2mM in the activity assay buffer.

In the Claims:

Claims 1-3 and 24 have been amended, as follows:

1. (Once Amended) A fusion protein comprising a first protease inhibitor comprising alpha 1-antitrypsin or a functionally active portion thereof, and a second protease inhibitor or a functionally active portion thereof, wherein said fusion protein has protease inhibitor activity.

2. (Once Amended) A fusion protein comprising alpha 1-antitrypsin or a functionally active portion thereof, and secretory leukocyte protease inhibitor or a functionally active portion thereof, wherein said fusion protein has protease inhibitor activity.

3. (Once Amended) A fusion protein comprising alpha 1-antitrypsin or a functionally active portion thereof, and a tissue inhibitor of metalloproteases or a functionally active portion thereof, wherein said fusion protein has protease inhibitor activity.

24. (Once Amended) A fusion protein comprising

a) a polypeptide comprising amino acids from about 1 to about 394 of alpha 1-antitrypsin; and

b) a polypeptide comprising amino acids from about 1 to 127 of tissue inhibitor of metalloproteases-1, wherein the alpha 1-antitrypsin polypeptide is covalently linked to the tissue inhibitor of metalloproteases-1 polypeptide through a disulfide bond between amino acid 127 of the tissue inhibitor of metalloproteases-1 polypeptide and a free cysteine residue of the alpha 1-antitrypsin polypeptide,

wherein said fusion protein has protease inhibitor activity.